

We Claim:

1. A method for transferring a nucleic acid fragment of interest from Parent Molecule 1 to Parent Molecule 2 wherein Parent Molecule 1 comprises the nucleic acid fragment of interest and Parent Molecule 2 comprises at least one functional element that is able to influence the transcription, translation or replication of the nucleic acid fragment of interest, or for transferring a nucleic acid fragment of interest from Parent Molecule 2 to Parent Molecule 1 wherein Parent Molecule 2 comprises the nucleic acid fragment of interest and Parent Molecule 1 comprises at least one functional element that is able to influence the transcription, translation or replication of the nucleic acid fragment of interest,

wherein Parent Molecule 1 may be linear or circular, and Parent Molecule 2 may be linear or circular;

wherein Parent Molecule 1 comprises a first recombination site and Parent Molecule 2 comprises a second recombination site;

wherein Parent Molecule 1 if linear comprises a first joinable end (JE1), and if circular comprises a first region (CS1) that can be converted into a first joinable end; and

wherein Parent Molecule 2 if linear comprises a second joinable (JE2) end and if circular comprises a second region (CS2) that can be converted into a second joinable end;

the method comprising:

- (1) mixing Parent Molecule 1 and Parent Molecule 2 *in vitro*;
- (2) forming an intermediate molecule, by either (a) site-specific recombination between the first and second recombination sites, or by (b)(i) joining the first and second joinable ends, or (ii) first converting the region or regions that can be converted into a joinable end or joinable ends, and then joining the joinable ends, the intermediate molecule comprising the nucleic acid molecule of interest and the at least one functional element; and
- (3) processing the intermediate respectively by either (a) joining the first and the second joinable ends, or (b) by site-specific recombination between the first and second recombination sites, to form a circular Product Vector comprising the nucleic acid molecule of interest and the at least one functional element.

2. The method according to Claim 1, wherein the method consists of one recombination reaction between two recombination sites.

3. The method according to Claim 1, wherein both Parent Molecules are linear, and the method consists of (1) first joining the two Parent Molecules to form an joined intermediate molecule, and then allowing joined intermediate molecule to undergo an intramolecular recombination; or (2) first allowing the two Parent Molecules to undergo a recombination to form two linear intermediate molecules

and then joining the two joinable ends now on one of the two linear intermediate molecules.

4. The method according to Claim 1, wherein at least one of the two Parent Molecules is circular, and the method consists of (1) allowing the two Parent Molecules to undergo site-specific recombination, (2) converting the CS1 or CS2 or both into joinable ends, and (3) joining the joinable ends.

5. The method according to Claim 1, wherein at least one of the two Parent Molecules is circular, and the method consists of (1) converting the CS1 or CS2 or both into joinable ends, forming two linear intermediate molecules, (2) allowing the two linear intermediate molecules to undergo site-specific recombination, and (3) joining the joinable ends.

6. The method according to Claim 1, wherein at least one of the two Parent Molecules is circular, and the method consists of (1) converting the CS1 or CS2 or both into joinable ends, forming two linear intermediate molecules, (2) joining the joinable ends, and (3) allowing the two linear intermediate molecules to undergo site-specific recombination.

7. A method according to Claim 1, wherein all reagents for at least two of the steps (1) the site-specific recombination, (2) converting the region or regions that can be converted into a joinable end or joinable ends, and (3) joining the joinable ends are added to one reaction vessel together.

8. A method according to Claim 1, wherein an end of a linear Parent Molecule or an intermediate other than JE1 or JE2 is deactivated and cannot be joined by the joining reaction.

9. A method according to Claim 8, wherein an end of a linear Parent Molecule or an intermediate other than JE1 or JE2 is deactivated by dephosphorylation.

10. A method according to Claim 1, wherein the functional element comprises at least one member selected from the group consisting of a promoter, a selection marker, a replication origin, a ribosome binding site, a transcription terminator, a C-terminal, a N-terminal tag, and a protease cleavage site.

11. A method according to Claim 1, wherein the joinable end is an end produced by cleavage with at least one restriction enzyme, a blunt end, an end with protruding single stranded 5' or 3' bases of sufficient length to anneal to a partner joinable end with complementary or substantially complementary single stranded bases, or an end comprising a covalently attached protein that can join the end to a suitable partner joinable end.

12. A method according to Claim 1, wherein the recombination site is selected from the group consisting of attB(wt), attP(wt), attL(wt), attR(wt), loxP(wt), frt(wt) or a derivative thereof.

13. A method according to Claim 12, wherein the recombination site is selected from the group consisting of attB1, attB2, attB3, attP1, attP2, attP3, attL1, attL2, attL3, attR1, attR2, or attR3.

14. A method of claim 1, further comprising

contacting one or more host cells with the desired circular Product Vector; and

selecting for a host cell comprising said circular Product Vector.

15. A method according to Claim 1, wherein in the Product Vector the nucleic acid fragment is downstream of the functional element, and wherein a recombination recognition site is not located downstream of the functional element and upstream of the nucleic acid fragment.

16. A method according to Claim 15, wherein the at least one functional element is a translational signal and the nucleic acid fragment encodes a polypeptide.

17. A method according to Claim 16, wherein the translational signal and the first translated ATG codon in the product vector are separated by not more than 12 nucleotides.

18. A method according to Claim 1, wherein both Parent Molecule 1 and Parent Molecule 2 are linear, wherein Parent Molecule 1 comprises the nucleic acid

fragment of interest and Parent Molecule 2 comprising the at least one functional element, and wherein

Parent Molecule 1 and Parent Molecule 2 are first incubated in the presence of at least one recombination protein under conditions sufficient to cause recombination of the first and second recombination sites, thereby producing a linear recombination product molecule having two ends and comprising the nucleic acid fragment of interest and the at least one functional element, the two ends of the linear recombination product molecule being the first joinable end and the second joinable end; and

the linear recombination product molecule is incubated in the presence of at least one ligation protein under conditions sufficient to cause joining of the first and second joinable ends, thereby producing the circular Product Vector.

19. A method according to Claim 1, wherein both the Parent Molecule 1 and Parent Molecule 2 are linear, wherein Parent Molecule 1 comprises the nucleic acid fragment of interest and Parent Molecule 2 comprising at least one functional element, and wherein

Parent Molecule 1 and Parent Molecule 2 are first incubated under conditions sufficient to cause joining of the first and second joinable ends, thereby producing a linear joined product comprising the first and second recombination sites, and the nucleic acid fragment of interest and the at least one functional element; and

the linear joined product is incubated in the presence of at least one recombination protein under conditions sufficient to cause recombination of the first and second recombination sites, thereby producing a circular Product Vector.

20. A method according to Claim 1, wherein Parent Molecule 1 is linear and Parent Molecule 2 is circular, wherein Parent Molecule 1 comprises the nucleic acid fragment of interest and Parent Molecule 2 comprising at least one functional element, and wherein

Parent Molecule 1 and Parent Molecule 2 are first incubated in the presence of at least one recombination protein under conditions sufficient to cause recombination of the first and second recombination sites, thereby producing a linear recombination product molecule having two ends, and comprising the nucleic acid fragment of interest and the at least one functional element, and one of the two ends of the linear recombination product molecule being the first joinable end;

the linear recombination product is further incubated in the presence of at least one restriction enzyme under conditions sufficient to cause the region that can be converted into a second joinable end to be converted into the second joinable end, thereby producing a linear digestion product having two ends and comprising the nucleic acid fragment of interest and the at least one functional element, the two ends of the linear recombination product molecule being the first joinable end and the second joinable end, and

the linear digestion product molecule is incubated under conditions sufficient to cause joining of the first and second joinable ends, thereby producing the circular Product Vector.

21. A method according to Claim 1, wherein Parent Molecule 1 is linear and Parent Molecule 2 is circular, wherein Parent Molecule 1 comprises the nucleic acid fragment of interest and Parent Molecule 2 comprising at least one functional element suitable for the expression of the nucleic acid fragment of interest, and wherein

Parent Molecule 2 is first incubated in the presence of at least one restriction enzyme under conditions sufficient to cause the region that can be converted into a second joinable end to be converted into the second joinable end, thereby producing a linear digestion product having a second joinable end,

the linear digestion product and Parent Molecule 1 are then incubated under conditions sufficient to cause joining of the first and second joinable ends, thereby producing a linear ligation product comprising the nucleic acid fragment of interest, the at least one functional element and the first and second recombination sites,

the linear ligation product is further incubated in the presence of at least one recombination protein under conditions sufficient to cause recombination of the first and second recombination sites, thereby producing the circular product vector.

22. A method according to Claim 1, wherein Parent Molecule 1 is circular and comprises the nucleic acid fragment of interest, and Parent Molecule 2 is linear and comprises the second joinable end and at least one functional element, and wherein

the first and second vectors are first incubated in the presence of at least one recombination protein under conditions sufficient to cause recombination of the first and second recombination sites, thereby producing a linear recombination product molecule having two ends and comprising the nucleic acid fragment of interest and the at least one functional element, and one of the two ends of the linear recombination product molecule being the second joinable end;

the linear recombination product is further incubated in the presence of at least one restriction enzyme under conditions sufficient to cause the first region that can be converted into a first joinable end, thereby producing a linear digestion product having two ends and comprising the nucleic acid fragment of interest and the at least one functional element, the two ends of the linear recombination product molecule being the first joinable end and the second joinable end, and

the linear digestion product molecule is incubated under conditions sufficient to cause joining of the first and second joinable ends, thereby producing the circular Product Vector.

23. A method according to Claim 1, wherein Parent Molecule 1 is linear and Parent Molecule 2 is circular, wherein Parent Molecule 2 comprising the nucleic acid fragment of interest, wherein Parent Molecule 1 comprises at least one functional element, and wherein

Parent Molecule 2 is first incubated in the presence of at least one restriction enzyme under conditions sufficient to cause the region that can be converted into a second joinable end, thereby producing a linear digestion product having a second joinable end,

the linear digestion product and Parent Molecule 1 are then incubated under conditions sufficient to cause joining of the first and second joinable ends, thereby producing a linear ligation product comprising the nucleic acid fragment of interest, the at least one functional element and the first and second recombination sites,

the linear ligation product is further incubated in the presence of at least one recombination protein under conditions sufficient to cause recombination of the first and second recombination sites, thereby producing the circular Product Vector.

24. A method according to Claim 1, wherein both Parent Molecule 1 and Parent Molecule 2 are circular, and wherein

Parent Molecule 1 and Parent Molecule 2 are first incubated in the presence of at least one recombination protein under conditions sufficient to cause recombination of the first and second recombination sites, thereby producing a circular recombination product molecule comprising the nucleic acid fragment of interest and the at least one functional element, and the first and second regions that can be converted respectively into the first and second joinable ends;

the circular recombination product is further incubated in the presence of at least one restriction enzyme under conditions sufficient to cause the first region that can be converted into a first joinable end, and the second region that can be converted into a second joinable end, thereby producing a linear digestion product having two ends and comprising the nucleic acid fragment of interest and the at least one functional element, the two ends of the linear recombination product molecule being the first joinable end and the second joinable end, and

the linear digestion product molecule is incubated under conditions sufficient to cause joining of the first and second joinable ends, thereby producing the circular Product Vector.

25. A nucleic acid molecule comprising the sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 11.

26. An oligonucleotide molecule of Claim 25, comprising SEQ ID NO: 3, wherein NNN represents a non-stop codon.

27. An oligonucleotide molecule of Claim 26, wherein NNN is GCG or GGT.

28. A method for amplifying a nucleic acid fragment of interest, wherein the 5'-end sequence and the 3'-end sequence of the nucleic acid fragment are known, the method comprising:

providing a first PCR primer comprising SEQ ID NO: 1 or SEQ ID NO: 3, linked immediately upstream to the 5'-end sequence of the nucleic acid fragment, and a second PCR primer comprising SEQ ID NO: 2 linked immediately upstream to the 5'-end sequence of the complementary strand of the nucleic acid fragment,

amplifying the nucleic acid molecule using the first and second primers under conditions suitable for amplification by the polymerase chain reaction, producing a first amplified product.

29. A method for amplifying a nucleic acid fragment of interest according to Claim 28, further comprising:

providing a third primer comprising SEQ ID NO: 1 or SEQ ID NO: 3 and a fourth primer comprising SEQ ID NO: 2,

amplifying the first amplified amplification product using the third and fourth primers under conditions suitable for amplification by the polymerase chain reaction, producing a second amplified product that comprises the first amplified amplification product flanked by the first and second linker sequences.

30. A method according to Claim 29, wherein the first, second, third and fourth primers are added together in one reaction vessel before the first amplified product is produced.

31. A method for producing a vector capable of expression a nucleic acid fragment of interest, comprising integrating the second amplification product of Claim 30 into a receiving vector.

32. The method of Claim 31, wherein the receiving vector comprises at least one member selected from the group consisting of a promoter, a replication origin, a positive selection marker gene, and a negative selection marker gene.

33. A method according to Claim 28, wherein the first PCR primer comprises SEQ ID NO: 3, and wherein NNN represents a non-stop codon.

34. A method according to Claim 33, wherein NNN is CGT or GCG.

35. A kit for transferring a nucleic acid fragment of interest from Parent Molecule 1 to Parent Molecule 2 according to a method according to Claim 1, wherein the kit comprises at least a Parent Molecule 1 and a Parent Molecule 2.